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TITLE: Method for indentifying or characterizing properties of polymeric units

Brief Summary Text (11):
In one embodiment the step of determining a property of the sample polymer involves the use of mass spectrometry, such as for example, matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), electron spray-MS, fast atom bombardment mass spectrometry (FAB-MS) and collision-activated dissociation mass spectrometry (CAD) to determine the molecular weight of the polymer. MALDI-MS, for instance, may be used to determine the molecular weight of the polymer with an accuracy of approximately one Dalton.

Brief Summary Text (17):
The properties of the modified polymer may be detected in any manner possible which depends on the property and polymer being analyzed. In one embodiment the step of detection involves mass spectrometry such as matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), electron spray MS, fast atom bombardment mass spectrometry (FAB-MS) and collision-activated dissociation mass spectrometry (CAD). Alternatively, the step of detection involves strong ion exchange chromatography, for example, if the polymer has been digested into several smaller fragments composed of several units each.

Brief Summary Text (21):
Similarly to the aspects of the invention described above the properties of the polymer may be detected in any manner possible and will depend on the particular property and polymer being analyzed. In one embodiment the step of detection involves mass spectrometry such as matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), electron spray MS, fast atom bombardment mass spectrometry (FAB-MS) and collision-activated dissociation mass spectrometry (CAD). Preferably the experimental constraint applied to the polymer is an enzymatic or chemical reaction which involves incomplete enzymatic digestion of the polymer and wherein the steps of the method are repeated until the number of polymers within the reference database falls below a predetermined threshold: Alternatively, the step of detection involves capillary electrophoresis, particularly when the experimental constraint applied to the polymer involves complete degradation of the polymer into individual chemical units.

Brief Summary Text (24):
In another aspect the invention relates to a method for identifying a polysaccharide-protein interaction, by contacting a protein-coated MALDI surface with a polysaccharide containing sample to produce a polysaccharide-protein-coated MALDI surface, removing unbound polysaccharide from the polysaccharide-protein-coated MALDI surface, and performing MALDI mass spectrometry to identify the polysaccharide that specifically interacts with the protein coated on the MALDI surface.

Brief Summary Text (25):
In one embodiment a MALDI matrix is added to the polysaccharide-protein-coated MALDI surface. In other embodiments an experimental constraint may be applied to the polysaccharide bound on the polysaccharide-protein-coated MALDI surface before performing the MALDI mass spectrometry analysis. The experimental constraint applied to the polymer in some embodiments is digestion with an exoenzyme or digestion with an endoenzyme. In other embodiments the experimental constraint applied to the polymer is

selected from the group consisting of restriction endonuclease digestion; chemical digestion; chemical modification; and enzymatic modification.

Drawing Description Text (9):
FIG. 8 is a graph depicting MALDI-MS analysis of the extended core structures derived from enzymatic treatment of a mixture of bi- and triantennary structures.

Drawing Description Text (10):
FIG. 9 is a graph depicting MALDI-MS analysis of the PSA polysaccharide. (A) intact polysaccharide structure. (B) Treatment of [A] with sialidase from A. urefaciens. (C) Digest of [B] with galactosidase from S. pneumoniae. (D) Digest of [C] with N-acetylhexosaminidase from S. pneumonia. (E) Table of the analysis scheme with schematic structure and theoretical molecular masses. [.largecircle.]=mannose; [.star-solid.]=fucose; [{character pullout}]=N-acetylglucosamine; [.quadrature.]=galactose; and [.DELTA.]=N-acetylneuraminic acid. Peaks marked with an asterisk are impurities, and the analyte peak is detected both as M-H (m/z 2369.5) and as a monosodiated adduct (M+Na-2H, m/z 2392.6).

Detailed Description Text (19):
The property of the polymer may be identified by any means known in the art. The procedure used to identify the property will depend on the type of property. Molecular weight, for instance, may be determined by several methods including mass spectrometry. The use of mass spectrometry for determining the molecular weight of polymers is well known in the art. Mass Spectrometry has been used as a powerful tool to characterize polymers because of its accuracy (.+-.1 Dalton) in reporting the masses of fragments generated e.g. by enzymatic cleavage and also because only pM sample concentrations are required. For instance matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) has been described for identifying the molecular weight of polysaccharide fragments in publications such as Rhomberg, A. J. et al, PNAS, USA, v. 95, p. 4176-4181 (1998); Rhomberg, A. J. et al, PNAS, USA, v. 95, p. 12232-12237 (1998); and Ernst, S. et. al., PNAS, USA, v. 95, p. 4182-4187 (1998), each of which is hereby incorporated by reference. Other types of mass spectrometry known in the art, such as, electron spray-MS, fast atom bombardment mass spectrometry (FAB-MS) and collision-activated dissociation mass spectrometry (CAD) may also be used to identify the molecular weight of the polymer or polymer fragments.

Detailed Description Text (29):
Methods of identifying other types of properties may be easily identifiable to those of skill in the art and may depend on the type of property and the type of polymer. For example, hydrophobicity may be determined using reverse-phase high-pressure liquid chromatography (RP-HPLC). Enzymatic sensitivity may be identified by exposing the polymer to an enzyme and determining a number of fragments present after such exposure. The chirality may be determined using circular dichroism. Protein binding sites may be determined by mass spectrometry, isothermal calorimetry and NMR. Enzymatic modification (not degradation) may be determined in a similar manner as enzymatic degradation, i.e., by exposing a substrate to the enzyme and using MALDI-MS to determine if the substrate is modified. For example, a sulfotransferase may transfer a sulfate group to an HS chain having a concomitant increase in 80 Da. Conformation may be determined by modeling and nuclear magnetic resonance (NMR). The relative amounts of sulfation may be determined by compositional analysis or approximately determined by Raman spectroscopy.

Detailed Description Text (33):
Nitrous acid degraded fragments, unlike heparinase-derived fragments, do not have a UV-absorbing chromophore. As we have shown, MALDI-MS will record the mass of heparin fragments regardless of how they are derived. For CE, two methods may be used to monitor fragments that lack a suitable chromophore. First is indirect detection of fragments. We may detect heparin fragments with our CE methodology using a suitable background absorber, e.g., 1,5-napthalenedisulfonic acid. The second method for detection involves chelation of metal ions by saccharides. The saccharide-metal complexes may be detected using UV-Vis just like monitoring the unsaturated double bond.

Detailed Description Text (34):
Other groups have begun the process of raising antibodies to specific HLGAG sequences.

We have previously shown that proteins, e.g., angiogenin, FGF, may be used as the complexing agent instead of a synthetic, basic peptide. By extension, antibodies could be used as a complexing agent for MALDI-MS analysis. This enables us to determine whether specific sequences are present in an unknown sample simply by observing whether a given antibody with a given sequence specificity complexes with the unknown using MALDI-MS.

Detailed Description Text (67):
In addition to the use of the methods of the invention for sequencing polymers, the methods may be used for any purpose in which it is desirable to identify structural properties related to a polymer. For instance the methods of the invention may be used for analysis of low molecular weight heparin. By limited digestion of LMWH and analysis by CE and MALDI-MS, we may obtain an "digest spectrum" of various preparations of LMWH, thus deriving information about the composition and variations thereof. Such information is of value in terms of quality control for LMWH preparations.

Detailed Description Text (69):
Additionally the methods of the invention are useful for mapping protein binding HLGAG sequences. Analogous to fingerprinting DNA, the MALDI-MS sequencing approach may be used to specifically map HLGAG sequences that bind to selected proteins. This is achieved by sequencing the HLGAG chain in the presence of a target protein as well as in the absence of the particular protein. In this manner, sequences protected from digestion are indicative of sequences that bind with high affinity to the target protein.

Detailed Description Text (77):
A mass line of the 256 different polysaccharide structures may be plotted. Then the rules of biosynthetic pathways may be used to further analyze the polysaccharide. In the example (shown in FIG. 4B), it is known that the first step of the biosynthetic pathway is the addition of GlcNac at the 1-3 linked chain (branch 1). Thus, branch 1 should be present for any of the other branches to exist. Based on this rule the 256 possible combinations may be reduced using a factorial approach to conclude that the branch 2, 3, and 4 exist if and only if branch one is non-zero. Similar constraints can be incorporated at the notation level before generation of the master list of ensembles. With the notation scheme in place, experimental data can be generated (such as MALDI-MS or CE or chromatography) and those sequences that do not satisfy this data can be eliminated. An iterative procedure therefore enables a rapid convergence to a solution.

Detailed Description Text (78):
To identify branching patterns, a combination of MALDI-MS and CE (or other techniques) may be used, as shown in the Examples. Elimination of the pendant arms of the branched polysaccharide may be achieved by the judicious use of exo and endoenzymes. All antennary groups may be removed, retaining only the GlcNAc moieties extending from the mannose core and forming an "extended" core. In this way, information about branching is retained, but separation and identification of glycoforms is made simpler. One methodology that could be employed to form extended cores for most polysaccharide structures is the following. Addition of sialidases, and fucosidases will remove capping and branching groups from the arms. Then application of endo-.beta.-galactosidase will cleave the arms to the extended core. For more unusual structures, other exoglycosidases are available, for instance xylases and glucosidases. By addition of a cocktail of degradation enzymes, any polysaccharide motif may be reduced to its corresponding "extended" core. Identification of "extended" core structures will be made by mass spectral analysis. There are unique mass signatures associated with an extended core motif depending on the number of pendant arms (FIG. 4D). FIG. 4D shows a massline of the "extended" core motifs generated upon exhaustive digest of glycan structures by the enzyme cocktail. Shown are the expected masses of mono-, di-, tri- and tetrantennary structures both with and without a fucose linked .alpha.1.fwdarw.6 to the core GlcNAc moiety (from left to right). All of the "extended" core structures have a unique mass signature that is easily resolved by MALDI MS (from left to right). Quantification of the various glycan cores present may be completed by capillary electrophoresis, which has proven to be a highly rapid and sensitive means for quantifying polysaccharide structures. [Kakehi, K. and S. Honda, Analysis of glycoproteins, glycopeptides and glycoprotein-derived

polysaccharides by high-performance capillary electrophoresis. J Chromatogr A, 1996. 720(1-2): p. 377-93.]

Detailed Description Text (99):
MALDI-MS of a basic fibroblast growth factor (FGF-2) binding saccharide was performed to determine the mass and size of the saccharide as a complex with FGF-2 (G. Venkataraman et al., PNAS. 96, 1892, (1999)). Dimers of FGF-2 bound to the saccharide (S) yielding a species with a m/z of 37,009. By subtraction of FGF-2 molecular weight, the molecular mass of the saccharide was determined to be 2808, corresponding to a decasaccharide with 14 sulfates and an anhydromannitol at the reducing end.

Detailed Description Text (109):
Compositional analysis and CE were completed as described above. Compositional analysis of an AT-III binding saccharide indicated the presence of three building blocks, corresponding to .DELTA.U.sub.2S H.sub.NS,6S (.+-.D), .DELTA.UH.sub.NAc,6S (.+-.4) and .DELTA.UH.sub.NS,3S,6S (.+-.7) in the relative ratio 3:1:1 respectively. The shortest polysaccharide that may be formed with this composition corresponds to a decasaccharide, consistent with the MALDI-MS data. The total number of possible combinations of this tridecasulfated single acetylated decasaccharide sequences with the above disaccharide building blocks is 320 Table 6. ##STR3##

Detailed Description Text (125):
The sequencing approach may be readily extended to other complex polysaccharides by developing appropriate experimental constraints. For example, the dermatan/chondroitin mucopolysaccharides (DCMP) consisting of a disaccharide repeat unit is amenable to a hexadecimal coding system and MALDI-MS. Similar to what is observed for HLGAGs, there is unique signature associated with length and composition to a given mass in DCMP. For instance, the minimum difference between any disaccharide and any tetrasaccharide is 139.2 Da, therefore, the length, the number of sulfates and acetates may be readily assigned for a given DCM polysaccharide up to an octa-decasaccharide. Similarly, in the case of polysialic acids (PSA), present mostly as homopolymers of 5-N-acetylneuraminic acid (NAN) or 5-N-glycolylneuraminic acid (NGN), the hexadecimal coding system may be easily extended to NAN/NGN to encode the variations in the functional groups and enabling a sequencing approach for PSA.

Detailed Description Text (127):
DCMP are found in dense connective tissues such as bone and cartilage. The basic repeat unit of the dermatan/chondroitin mucopolysaccharides (DCMP) may be represented as -(.beta.1.fwdarw.4)U.sub.2X -(.alpha./.beta.1.fwdarw.3)Gal.sub.NAc, 4X, 6X.sub..sub.- , where U is uronic acid, Gal.sub.NAc is a N-acetylated galactosamine. The uronic acid may be glucuronic acid (G) or iduronic acid (I) and sulfated at the 2-O position and the galactosamine (GaINAc) may be sulfated in the 4-O or the 6-O position, thereby resulting in 16 possible combinations or building blocks for DCMP. Like the heparinases that degrade HLGAGs, there are distinct chondoroitinases and other chemical methods available that clip at specific glycosidic linkages of DCMP and serve as experimental constraints. Furthermore, since DCMPs are acidic polysaccharides, the MALDI-MS techniques and methods used for HLGAGs may be readily extended to the DCMPs.

Detailed Description Text (145):
Secondary specificities of the heparinases have been observed, especially under exhaustive degradation conditions. As a part of ongoing investigations into the enzymology of heparinases, the relative rates of cleavage of I and G containing sites by heparinase I and III with defined substrates under different conditions have been measured. For instance heparinase III cleaves both at I and G containing linkages and not I.sub.2S [H. E. Conrad, Heparin Binding Proteins (Academic Press, San Diego, 1998).]. However, under the reaction conditions used in this study, there is a dramatic (8-10 fold) difference in the rates of cleavage, with I-containing linkages being clipped more slowly than G-containing linkages (FIG. 7A). FIG. 7A shows cleavage by recombinant heparinase III of tetrasaccharides containing either G (.circle-solid.), I(.largecircle.) or I.sub.2S (.diamond-solid.) linkages. Each reaction was followed by capillary electrophoresis. With these substrates, heparinase III does not cleave I.sub.2S -containing glycosidic linkages, and cleaves G-containing linkages roughly 10 times as fast as I-containing linkages. Under the "short" conditions of digest it is expected that only G-containing saccharides are cleaved to

an appreciable extent. [Conditions for enzymatic digest of HLGAG oligosaccharides were set forth above, briefly, Digests were either designated as "short" or "exhaustive". Short digests were completed with 50 nM enzyme for 10 minutes. Exhaustive digests were completed using 200 nM enzyme for either four hours or overnight. Partial nitrous acid cleavage was completed using a modification of published procedures. Briefly, to an aqueous solution of saccharide was added a 2.times.solution of sodium nitrite in HCl such that the concentration of nitrous acid was 2 mM and HCl was 20 mM. The reaction was allowed to proceed at room temperature with quenching of aliquots at various time points via the addition of 1 .mu.L of 200 mM sodium acetate 1 mg/mL BSA pH 6.0. Exhaustive nitrous acid was completed by reacting saccharide with 4 mM nitrous acid in HCl overnight at room temperature. In both cases, it was found that the products of nitrous acid cleavage could be sampled directly by MALDI without further cleanup and without the need to reduce the anhydromannose residues to anhydromannitol. The entire panel of HLGAG degrading exoenzymes were purchased from Oxford Glycosystems (Wakefield, Mass.) and used as suggested by the manufacturer.] For example, with the hexasaccharide .DELTA.UH.sub.NH,6S GH.sub.NS IH.sub.NAc, (which contains both I and G in a minimally sulfated region) cleavage occurs only at the G under "short" digest conditions as shown in Table II.

Detailed Description Text (147):
Quite a few factors have severely limited and complicated prior art studies and interpretation of heparinase substrate specificity experiments. First, not only is a homogenous substrate preparation difficult, but also analyzing the substrates and products have been very challenging. Analysis has primarily relied on co-migration of the saccharides with known standards, and as others and we have observed, oligosaccharides with different sulfation patterns do co-migrate, complicating unique assignments. Further, some oligosaccharides used in previous studies to assign substrate specificity for the heparinases were not homogeneous, complicating analysis. The development of the MALDI-MS procedure of the invention has enabled rapid and accurate determination of the saccharides. The second problem is the preparation of pure wild-type heparinases from the native host. The wild-type heparinase is isolated from Flavobacterium heparinum and this organism produces several complex polysaccharide-degrading enzymes, and often these copurify with each other. For example, when examining the kinetics of heparinase III, we found that a commercial source of heparinase III was able to degrade the supposedly non-cleavable .DELTA.U.sub.2S H.sub.NS,6S I.sub.2S H.sub.NS,6S. Furthermore, MS and CE analysis of the products indicated that one was specifically 2-O desulfated suggesting a sulfatase contamination. Recombinant heparinase III produced and purified in our laboratory (and not having contamination with other heparin degrading enzymes) does not cleave .DELTA.U.sub.2S H.sub.NS,6S I.sub.2S H.sub.NS,6S as expected. Thus, different enzyme preparations and differences in digestion conditions, and differences in substrate size and composition and often contaminating substrates, taken together with assignments based on co-elution make comparison of data not only very difficult but also has led to contradictory findings.

Detailed Description Text (149):
While there are caveats to the use of any one particular system for sequence analysis, whether the system is chemical degradation or enzymatic analysis, the sequencing strategy presented here is not critically dependent on any, single technique. One of the major strengths of the sequencing strategy of the invention is the flexibility of our approach and the integration of MALDI and the coding scheme which enable the ability to adapt to different experimental constraints [For example, the recently cloned mammalian heparanase is another possible experimental constraint. M. D. Hulett et al., Nat. Med. 5,793 (1999); I. Vlodavsky et. al., Nat. Med. 5, 803 (1999).]. As stated additional or different sets of experimental constraints may be used to not only arrive at a unique solution but also may be used to validate or confirm the solution from a given set of experimental constraints.

Detailed Description Text (156):
Protein preparation and immobilization. ATIII was incubated.overnight with excess porcine mucosal heparin, then biotinylated with EZ-link sulfo-NHS biotin (Pierce). Canon NP Type E transparency film was taped to the MALDI sample plate and used as a protein immobilization surface. FGF-1 and FGF-2 were immobilized by spotting 1 .mu.l of aqueous solution on the film and air-drying. ATIII was immobilized by first drying 4 .mu.g neutravidin on the film surface, then adding biotinylated ATIII to the

neutravidin spot. Heparin was removed by washing ten times with 1M NaCl and ten times with water.

Detailed Description Text (157):
Saccharide binding, selection and analysis. Saccharides were derived from a partial digest of porcine mucosal heparin by heparinase I. The hexasaccharide fraction was obtained by size exclusion chromatography on Biogel P-6 and lyophilized to dryness. Saccharides were bound to immobilized proteins by spotting 1 .mu.l of aqueous solution on the protein spot for at least five minutes. Unbound saccharides were removed by washing with water fifteen times. For selection experiments, the spot was washed ten times with various NaCl concentrations, followed by ten water washes. Caffeic acid matrix in 50% acetonitrile with 2 pmol/.mu.l (RG).sub.19 R was added to the spot prior to MALDI analysis. All saccharides were detected as noncovalent complexes with (RG).sub.19 R using MALDI parameters described herein.

Detailed Description Text (158):
Saccharide digestion by heparinase I or III. Saccharides selected for FGF-2 binding were digested with heparinases I or III by spotting 8 .mu.g of enzyme in water after selection was completed. The spot was kept wet for the desired digestion time by adding water as necessary. Caffeic acid matrix with 2 pmol/.mu.l (RG).sub.19 R was added to the spot for MALDI analysis.

Detailed Description Text (161):
Saccharide binding to FGF-2 and FGF-1. As a first step towards the development of a viable MALDI selection procedure, the FGF system using its prototypic members, viz. FGF-1 and FGF-2 was selected. Initial experiments involved the use of a purified polysaccharide (Hexa 1 of Table 12) that is known to bind with high affinity to FGF. With FGF-2, we found that Hexa 1 binds to FGF-2 and were detected, even with a salt wash of 0.5M NaCl, consistent with the known affinity of Hexa 1 for FGF-2. In addition, when an equimolar mixture of Hexa 1 and Hexa 2 (a low affinity binder) were applied to FGF-2 and washed with 0.2M NaCl to eliminate nonspecific binding, only Hexa 1 was observed. Together, these results point to the fact that, under of the conditions of the experiment, immobilized FGF-2 retained the same binding specificity as FGF in solution. Further demonstrating that binding specificity was intact, heat denaturation of FGF resulted in the detection of no saccharide binders.

Detailed Description Text (163):
Sequencing saccharides on the MALDI surface. The highly sensitive sequencing methodology of the invention was used to test whether we could derive structural information of FGF high affinity binders on target. The octa- and nonasulfated saccharides were subjected to enzymatic and chemical depolymerization. After saccharide selection, the saccharide sample was depolymerized by heparinase I to obtain sequence information. The nonasulfated hexasaccharide was reduced to a single trisulfated disaccharide indicating that this saccharide is a repeat of [I.sub.2S H.sub.NS,6S ]. Digestion of the octasulfated hexasaccharide yielded the trisulfated disaccharide and a pentasulfated tetrasaccharide. That this tetrasaccharide contains an unsulfated uronic acid was confirmed by heparinase III cleavage, which resulted in the disappearance of the tetrasaccharide. Confirmation of our sequencing assignments were made by isolating the octa- and nonasulfated hexasaccharides and sequenced using the methods described herein. Thus, the sequence of the nonasulfated hexasaccharide is .+-.DDD (.DELTA.U.sub.2S H.sub.NS,6S I.sub.2S H.sub.NS,6S I.sub.2S H.sub.NS,6S) and the sequence of the octasulfated hexasaccharide is .+-.DD-5.

Detailed Description Text (164):
Saccharide Binding to Antithrombin-III. ATIII is heavily glycosylated, therefore we anticipated that it would not bind well to the MALDI plate. As an alternative strategy, avidin was immobilized on the plate and biotinylated AT-III was bound to the avidin. The ATIII biotinylation reaction was carried out in the presence of heparin to protect the protein's binding site for HLGAG oligosaccharides. After washing off the complexed heparin, penta 1, that contains an intact AT-III pentasaccharide binding sequence was used to verify that the protein was immobilized on the surface and was able to bind saccharides. Penta 1 binding to ATIII was observed up to washes of 0.5M NaCl, consistent with it being a strong binder to ATIII.

Detailed Description Text (172):

MS Analysis of Complex Glycan Structures: As shown in FIG. 8, the extended core structures generated from complex N-glycan structures were enzymatically generated and identified. MALDI-MS analysis was performed on the extended core structures derived from enzymatic treatment of a mixture of bi- and triantennary structures. 1 pmol of each saccharide was subjected to digest with an enzyme cocktails that included sialidase from A. urefaciens and .beta.-galactosidase from S. pneumonia. The mass signature of 1462.4 indicates that one of the structures is biantennary with a core fucose moiety, while the mass signature of 1665.8 is indicative of a triantennary structure, also with a core fucose. [.largecircle.]=mannose; [.star-solid.]=fucose; [{character pullout}]=N-acetylglucosamine; [.quadrature.]=galactose; and [.DELTA.]=N-acetylneuraminic acid.

Detailed Description Text (173):
MALDI-MS sequencing of the N-linked polysaccharide of PSA: Next, rapid sequencing of the glycan structure of PSA from normal prostate tissue was performed (FIG. 9). FIG. 9 is data arising from MALDI-MS microsequencing of the PSA polysaccharide structure. MALDI-MS was completed using 500 fmol of saccharide. Analysis was completed with a saturated aqueous solution of 2,5-dihydroxybenzoic with 300 mM spermine as an additive. Analytes were detected in the negative mode at an accelerating voltage of 22 kV. 1 .mu.L of matrix was added to 0.5 .mu.L of aqueous sample and allowed to dry on the target. (A) MS of the intact polysaccharide structure. Peaks marked with an asterisk are impurities, and the analyte peak is detected both as M-H (m/z 2369.5) and as a monosodiated adduct (M+Na-2H, m/z 2392.6). (B) Treatment of [A] with sialidase from A. urefaciens. 10 pmol of saccharide was incubated with enzyme overnight at 37.degree. C. in 10 mM sodium acetate pH 5.5 according to the manufacturer's instructions. Two new saccharides were seen, the first, at m/z 2078 corresponding to the loss of one sialic acid moiety and the second at m/z 1786.9 corresponding to the loss of two sialic acids from the non-reducing end. (C) Digest of [B] with galactosidase from S. pneumonia. Digest procedures were completed essentially as described above. A signal product at m/z 1462.8 indicated that two galactose residues were removed upon treatment of [B] with the enzyme. (D) Digest of [C] with N-acetylhexosaminidase from S. pneumonia. One product was observed as both M-H (m/z 1056.3) and M+Na-2H (m/z 1078.1) corresponding to the loss of two N-acetylhexosamine units from [C]. A Table of the analysis scheme with schematic structure and theoretical molecular masses is presented in the center of FIG. 9. Shown are the parent polysaccharide and enzymatically derived products seen in this analysis. [.largecircle.]=mannose; [.female.]=fucose; [{character pullout}]=N-acetylglucosamine; [.quadrature.]=galactose; and [.DELTA.]=N-acetylneuraminic acid.

Detailed Description Text (174):
Studies of the intact polysaccharide via NMR (large quantities of PSA were required for this study) yielded sequence information of the glycan [Belanger, A., van Halbeek, H., Graves, H. C. B., Grandbois, K., Stamey, T. A., Huang, L., Poppe, I., and Labrie, F., Prostate, 1995. 27: p. 187-197]. Similar to other N-linked glycoproteins, as stated above, PSA contains a core biantennary branched motif. Extending from each mannose arm of PSA is a trisaccharide unit. Together these modifications indicated an expected molecular mass of 2370 Da for the intact polysaccharide. Using MALDI-MS and an exoglycosidase array we have sequenced the putative structure for the N-linked polysaccharide on PSA (FIG. 9). Analysis of the intact polysaccharide yields a molecular mass of 2370 Da (FIG. 9A), identical to the predicted molecular mass based on its structure. In fact for all structures and enzymatic products derived from them, a mass accuracy of less than one Dalton is realized.

Detailed Description Text (176):
Direct Sequencing of the PSA Polysaccharide Information about the structure of the sugar moiety of PSA can not only be derived by isolating the sugar and sequencing it (such as by using the above methodology), but we can also derive information about the sugar structure without removal from the protein. FIG. 10 shows the results of sequencing the sugar of PSA (Sigma Chemical). FIG. 10 shows the results of enzymatic degradation of the saccharide chain directly off of PSA. 50 pmol of PSA (.about.1.4 .mu.g) of PSA was denatured by heat treatment at 80.degree. C. for 20 minutes. Then the sample was sequentially treated with the exoenzymes (B-D). After overnight incubation at 37.degree. C., 1 pmol of the digested PSA was examined by mass spectrometry. Briefly, the aqueous sample was mixed with sinapinic acid in 30% acetonitrile, allowed to dry, and then examined by MALDI TOF. All spectra were

calibrated externally with a mixture of myoglobin, ovalbumin, and BSA to ensure accurate molecular mass determination. (A) PSA before the addition of exoenzymes. The measured mass of 28,478 agreed well with the reported value of 28,470. (B) Treatment of (A) with sialidase resulted in a mass decrease of 287 Da, consistent with the loss of one sialic acid residue. (C) Treatment of (B) with galactosidase. A further decrease of 321 Da indicated the loss of two galactose moieties. (D) Upon digestion of (C) with hexosaminidase, a decrease of 393 Da indicated the loss of two N-acetylglucosamine residues.

CLAIMS:

1. A method for identifying a polysaccharide-protein interaction, comprising: contacting a protein-coated MALDI surface with a polysaccharide containing sample to produce a polysaccharide-protein-coated MALDI surface, removing unbound polysaccharide from the polysaccharide-protein-coated MALDI surface, and performing MALDI mass spectrometry to identify the polysaccharide that specifically interacts with the protein coated on the MALDI surface.

2. The method of claim 1, wherein a MALDI matrix is added to the polysaccharide-protein-coated MALDI surface.

3. The method of claim 1, further comprising applying an experimental constraint to the polysaccharide bound on the polysaccharide-protein-coated MALDI surface before performing the MALDI mass spectrometry analysis.